bio-protocol Preprint

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Cell lysis, IP, immunoblotting, and antibodies

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An abbreviated version of this protocol was published in Science Advances in Sep 2020

USP15 suppresses tumor immunity via deubiquitylation and inactivation of TET2

DOI: 10.1126/sciadv.abc9730

Detailed protocol

1. Cells were washed with ice-cold phosphate buffered saline (PBS)
2. Add NP-40 lysis buffer (with 1% proteinase inhibitor), cell were lysed at 4°C for 30 min
3. Centrifuge at maximum speed, 4°C, 10 min
4. Transfer supernatant to a new tube
5. Mix the samples with the antibody and beads and incubate at 4°C for 3 hours
6. Centrifuge at 2000 ×g, 1 min
7. Wash the beads with NP-40 buffer 3 times
8. Add the loading buffer and boil at 95°C for 10 min
9. Spin down the beads
10. SDS-PAGE

Solutions:
NP-40 Buffer: 50mM Tris-HCl ,150 mM NaCl, 0.3% NP-40
Antibodies:

Antibody	Concertation for IP	Concertation for WB	Company	Catalogue number
Flag	1:1000	1:3000	Sigma	Clone M2
HA	1:1000	1:3000	Roche	Clone 3F10
MYC	1:1000	1:3000	Roche	Clone 9E10
TET2	1:200	1:1000	Millipore	MABE462
USP15	1:200	1:1000	Abcam	ab71713
Tubulin	Not Tested	1:5000	Santa Cruz	sc-23948

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Xu, Y. (2021). Cell lysis, IP, immunoblotting, and antibodies. Bio-protocol Preprint. bio-protocol.org/prep903.
2. Chen, L., Smith, M. D., Lv, L., Nakagawa, T., Li, Z., Sun, S., Brown, N. G., Xiong, Y. and Xu, Y.(2020). USP15 suppresses tumor immunity via deubiquitylation and inactivation of TET2 . Science Advances 6(38). DOI: 10.1126/sciadv.abc9730

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